Appl. No.	:	10/764,978
Confirmation No.	:	9303
Applicant	:	Plamen Denchev
Filed	:	January 23, 2004
Title	:	METHODS FOR REPRODUCING CONIFERS BY SOMATIC EMBRYOGENESIS
TC/A.U.	:	1661
Examiner	:	Hwu, June
Docket No.	:	205502-9037-US00

I, Sally Sorensen, hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office on the date of my signature.

Sally Sorensen
Signature

May 16, 2008
Date of Signature

DECLARATION OF STEPHEN ATTREE
UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Stephen Attree, do hereby declare and state the following:

1. I am currently a Director of Intellectual Property at CellFor, Inc. ("CellFor"). I have been employed by CellFor since January 1, 2000.

2. I received a Bachelors of Science Degree in Botany from the University of Manchester, UK in 1982. I received a Ph.D. in Plant Biology from the University of Manchester in 1987.

3. Attached hereto as Exhibit A is a list of my relevant patents and publications.

4. I am a co-inventor of the subject matter of all claims pending in the above-identified patent application. I make this declaration in support of prosecution of the subject application before the U.S. Patent and Trademark Office ("USPTO").

5. I have read and understand the invention as disclosed in the above-identified patent application, including the invention described by the presently pending claims.

6. I have reviewed the Office Action of January 16, 2008. I understand that claims 1, 5-9, 12-13, 16-23, 27, 28, 33-34, and 36-43 are rejected under 35 U.S.C. § 103(a) as unpatentable over Attree (U.S. Patent No. 6,627,441) in view of Handley (U.S Patent No. 5,491,090). I also understand that claims 50-54 are rejected under 35 U.S.C. § 103(a) as unpatentable over Fan (U.S. Patent No. 6,689,609) in view of Handley. I also understand that claims 55-60 are rejected under 35 U.S.C. § 103(a) as unpatentable over Coke (U.S. Patent No. 5,534,433) in view of Pullman (U.S. Patent No. 6,492,174). I believe that the evidence presented herein demonstrates that the pending claims are not obvious in light of the cited references.

7. The pending claims are drawn to methods for reproducing coniferous somatic embryos by somatic embryogenesis comprising growing an embryogenic culture derived from an explant on a nutrient medium comprising lactose, lactose and an additional sugar or a galactose-containing sugar and an additional sugar in steps prior to the maturation step, namely the induction, maintenance and/or prematuration steps.

8. Induction, maintenance and prematuration are steps prior to maturation and the media used during these steps help the conifer cells to remain undifferentiated and to proliferate. The media used during induction, maintenance and prematuration generally contain a metabolizable carbon source, hormones such as auxin and/or cytokinin and have a low osmoticum. Maturation requires the cells to slow or stop proliferating and differentiate. Maturation media generally have no auxin or cytokinin, have ABA added and have a relatively high osmoticum. Germination requires further differentiation to form seedlings. Germination media also do not contain auxin or cytokinin and generally have a low osmoticum. Induction, maintenance and prematuration require the cells to proliferate and remain in an undifferentiated state, whereas maturation and germination require the cells to stop proliferating and differentiate. Because the goals at these different steps of the process are exactly opposite, the media used at different stages of the method are, and would be expected to be, distinct.

9. U.S. Patent No. 6,627,441 to Attree relates to methods of promoting maturation of embryos by increasing the water stress on the embryos during the maturation step. See

abstract. The Examiner suggests that Attree teaches use of lactose in prematuration medium at Table 5 and column 26, lines 25-38. Attree clearly indicates that the media in Table 5 which contain lactose are maturation media and not prematuration media. See column 26, lines 26-30 ("Thus, immature somatic embryos from suspension culture were...transferred to maturation medium containing 3% sucrose, 20μM ABA and adjusted to 290mmol/kg with PEG." Emphasis added). This first maturation medium represents week 1 in Table 5 and the medium was replaced weekly during maturation with the media indicated in Table 5. Thus, lactose was first added in the third week of maturation, not during prematuration as indicated by the Examiner.

10. In addition, Attree makes clear that the lactose in the maturation media was used as an osmoticum to increase the water stress. See column 26, lines 34-35 ("water potential was increased by adding lactose"). Thus, Attree was using lactose, not as a carbon source, but instead as a means of increasing the water stress on the cells. During maturation the increased water stress reduces the moisture content of the embryos, enhances lipid storage and enhances development into mature embryos capable of germination. These effects, while being important for maturation of the embryo, are the opposite of the desired effects during induction, maintenance and prematuration of the embryos. In induction, maintenance and prematuration, a low water stress (osmoticum) is desired. Thus, the disclosure of Attree that lactose could be used as an osmoticum in the maturation medium actually discouraged the use of lactose in the induction, maintenance or prematuration stages of somatic embrogenesis.

11. Prior to the results presented in the present application, lactose, a sugar found in milk and not generally available to plants, was not believed to be metabolized by plants. The fact that lactose could be used as a carbon source was unexpected as noted in the specification at least at page 6, lines 6-9, in Example 5, page 13-14 and Example 5.1, page 14. Without the knowledge that lactose could be used as a carbon source, there would be no reason to add lactose to the induction, maintenance or prematuration media.

12. In addition, before actually doing the experiments, we would not have predicted that use of lactose in the media during induction, maintenance and/or prematuration would have

such a beneficial effect in terms of producing somatic embryos as compared to sucrose or maltose. For example, Example 1 shows over 4 fold better induction for loblolly pine in a combination of lactose and glucose than in sucrose. Example 3 demonstrates about a 2 fold increase in somatic embryos per gram of tissue when lactose was used as the sole carbon source during maintenance of loblolly pine cultures. Similar results were obtained with Radiata Pine (Examples 6 and 7).

13. The superior results obtained using these methods could not have been predicted. Even if one would have thought that lactose or galactose would be effective carbon sources for use in induction, maintenance and prematuration, the results demonstrating much higher numbers of somatic embryos per gram of tissue were surprising. This represents a significant improvement in the field because maintenance and bulk-up of tissues is a large expense and by generating higher numbers of embryos per gram of tissue the costs of somatic embryogenesis can be decreased significantly. The unexpected benefits of using a galactose-containing sugar as compared to other more traditionally used sugars were noted in the specification at least at page 6, lines 23-25 and page 8, lines 15-21. These unexpected benefits seem to be generic to conifers as all three conifers tested demonstrated a significant improvement in the number of somatic embryos produced per gram of tissue when a galactose-containing sugar was used in induction, maintenance and/or prematuration media.

14. For the reasons set forth above in paragraphs 8-13, the results demonstrated in the Examples section of the present application are surprising and would not be expected based on the cited references.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 13 May 2008

Stephen Attree, Ph.D.

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Exhibit A

Relevant Patents and Publications: Stephen Attree, Ph.D.

Issued U.S. Patents

Attree, S.M. (2003). Increasing levels of growth regulator and/or water stressing during embryo development. United States Patent 6,627,441

Attree, S.M., Fowke, L.C. (2002). Desiccation tolerant Gymnosperm embryos. United States Patent 6,372,496

Attree, S.M., Fowke, L.C. (2002). Production of desiccation tolerant Gymnosperm embryos. United States Patent 6,340,581.

Attree, S.M., Fowke, L.C. (1999). Maturation, desiccation and encapsulation of gymnosperm somatic embryos. United States Patent 5,985,667.

Attree, S.M., Fowke, L.C. (1995). Desiccated conifer somatic embryos. United States Patent 5,464,769.

Patent applications

Ilic-Grubor, K., Attree, S.M., Fowke, L.C. Media and Methods for culturing plant embryos. PCT filed 1998.

Denchev, Attree, Kong, Tsai, Radley, Lobatcheval. A method for producing conifers by somatic embryogenesis using galactose containing compounds as a carbon and energy source. Filed 2003.

Lobatcheva, Attree, Liu and Williams. Bulk sorting of conifer somatic embryos. Filed 2003.

Rise, Grossnickle, Fan, Attree, Denchev, Krol, Shang. Aerated liquid priming of loblolly pine somatic embryos. Filed 2003.

Fan, Grossnickle, Rise, Attree, Folk. Method of ex vitro sowing, germination, growth and conversion of plant somatic embryos or germinants, and nutrient medium used therefore. Filed 2003

Kong, Denchev, Lobatcheva, Attree, Radley. Method of culturing conifer somatic embryos using S(+) abscisic acid. Filed 2005.

Refereed Journal Contributions

Research papers

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Attree, S.M., Pomeroy,M.K., Fowke, L.C. (1995). Development of white spruce (Picea glauca [Moench.] Voss) somatic embryos during culture with abscisic acid and omoticum, and their tolerance to drying and frozen storage. Journal of Experimental Botany 46: 433-439.

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Other Refereed Contributions

Refereed book chapters

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Sutton, B, Attree, S.M., El-Kassaby, Y., Cyr, D. (2000). Clonal propagation and tree improvement using somatic embryogenesis. TAPPI Journal .

Fowke, L.C., Attree, S.M. (1993). Applied and basic studies of somatic embryogenesis in white spruce (Picea glauca) and black spruce (Picea mariana). In; Woong Young Soh et al. (eds.), Advances in Developmental Biology and Biotechnology of Higher Plants. Korean Society of Plant Tissue Culture, Korea, pp. 5-17.

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Attree, S.M. (2001). Applications of micropropagation. Keynote presentation IAPTC June 2001, Saskatoon, Saskatchewan, Canada

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Fowke, L.C., Attree, S.M., Wang, H., Dunstan, D.I. (1990). Microtubule organization and cell division in embryogenic protoplast cultures of white spruce (Picea glauca). VIIth I.A.P.T.C meeting, June 24-29, Amsterdam, The Netherlands.

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